

Mucosal Tolerance Induction with Hypoallergenic Molecules in a Murine Model of Allergic Asthma

U. Wiedermann^a U. Herz^b S. Vrtala^a U. Neuhaus-Steinmetz^b H. Renz^b
C. Ebner^a R. Valenta^a D. Kraft^a

^aDepartment of Pathophysiology, University of Vienna, Austria; ^bClinical Chemistry and Molecular Diagnostic, Clinic of the Philipps University, Marburg, Germany

Key Words

Allergy, type I · Mucosal tolerance · Hypoallergenic molecules

Abstract

Type I allergy, frequently elicited by airborne allergens, has constantly increased within recent years. Birch pollen and its major allergen Bet v 1 represent a major source of type I allergens. By genetic engineering hypoallergenic Bet v 1 fragments were produced, which lost the IgE binding capacity but retained the T cell epitopes. We have established a murine model of aerosol sensitization to birch pollen and its major allergen Bet v 1, leading to type I allergic immune responses and airway hyperresponsiveness. In the present study we demonstrate that mucosal administration of recombinant Bet v 1 prior to sensitization led to allergen-specific suppression of B and T cell responses *in vivo* and *in vitro*, reduction of eosinophilic infiltration in the lungs and inhibition of airway hyperresponsiveness. Intranasal pretreatment with the nonanaphylactic fragments of Bet v 1 prevented allergic immune responses and airway inflammation to the same degree as the pretreatment with the com-

plete molecule. We conclude from our studies that mucosal tolerance induction with hypoallergenic molecules could provide a safe and convenient treatment strategy against type I allergies.

Copyright © 2001 S. Karger AG, Basel

Introduction

The prevalence of type I allergy has constantly increased within recent years. Among the population being afflicted by allergic diseases almost 25% are sensitized to birch pollen and its major allergen Bet v 1. Specific immunotherapy, performed by injecting increasing doses of allergen to induce allergen-specific hyporesponsiveness, is the treatment of choice in certain cases of pollinosis [1]. Even though this treatment is effective in the majority of patients it has several disadvantages, such as frequent injections, duration of the treatment over many years or anaphylactic reactions. A major concern in allergy research is to improve the therapeutic measures in order to enhance the safety and efficacy of the treatment combined with a better compliance of the patients.

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

© 2001 S. Karger AG, Basel
1018–2438/01/1243–0391\$17.50/0

Accessible online at:
www.karger.com/journals/iaa

Correspondence to: Dr. Ursula Wiedermann
Department of Pathophysiology, AKH, University of Vienna
Währinger Gürtel 18–20
A–1090 Vienna (Austria)
E-Mail ursula.wiedermann@akh-wien.ac.at

Allergen derivatives with reduced allergenicity have been suggested to be used for low risk immunotherapy. Such hypoallergenic molecules of the major birch pollen allergen Bet v 1 have recently been genetically produced [2, 3]. Two fragments of Bet v 1 (aa 1–74, aa 75–160), together covering the sequence of the whole molecule, displayed a 100-fold reduced capacity in inducing immediate-type skin reactions in birch pollen-allergic patients [4].

Changing the conventional parenteral route of administration to application via the mucosal surfaces is another strategy for improvement of specific immunotherapy. The first clinical trials suggest that sublingual immunotherapy can be effective [5]; the underlying mechanisms are, however, not yet precisely known.

Mucosal tolerance is a state of antigen-specific nonresponsiveness following mucosal application of a soluble antigen. Several experimental studies have shown that mucosal tolerance induction can be effective in suppressing Th1-mediated autoimmune diseases [6]. Fewer studies exist showing similar results in models of excessive Th2 cell activities, such as allergies [7].

We have established a murine model of aerosol sensitization to birch pollen and its major allergen Bet v 1, leading to allergic immune responses in vivo and in vitro [8]. Using this model we recently demonstrated that mucosal (nasal or oral) application of recombinant Bet v 1 prior to sensitization inhibited allergic sensitization at the B and T cell levels [8].

In the present study we compared the complete Bet v 1 molecule with the hypoallergenic fragments of Bet v 1 regarding mucosal tolerance induction. The results indicate that such hypoallergenic molecules could be promising candidates for mucosal allergy vaccines.

Material and Methods

Animals, Sensitization, Tolerization

Seven-week-old female BALB/c mice were used in the experiments. Sensitization was performed by one intraperitoneal injection of 1 µg recombinant (r)Bet v 1 adsorbed to Al(OH)₃, followed by an aerosol treatment with birch pollen extract as previously described [8].

Tolerization was done by three intranasal administrations of rBet v 1 (10 µg) or Bet v 1 fragments (10 µg) at 7 days' intervals prior to sensitization.

Determination of Antigen-Specific Antibodies

Microtiter plates were coated with rBet v 1 (2 µg/ml). Serum samples were diluted 1:1,000 for IgG1 and 1:20 for IgE. Rat anti-mouse IgG1 and IgE (1:1,000, Pharmingen) and thereafter peroxidase-conjugated mouse anti-rat IgG antibodies (1:1,000, Jackson) were used.

Type I Skin Tests

Birch pollen extract (2.5 µg/ml) and rBet v 1 (2.5 µg/ml) were injected intradermally into the shaved abdominal skin and the skin reactions evaluated as previously described [8].

Airway Reactivity

Measurements of airway responsiveness were performed 24 h after the last challenge by head-out body plethysmography as previously described [9]. Airflow signals were recorded in response to methacholine aerosolized with a nebulizer. The concentration of methacholine that caused a 50% reduction in expiratory airflow (MCh₅₀, mg/ml) was determined.

Results and Discussion

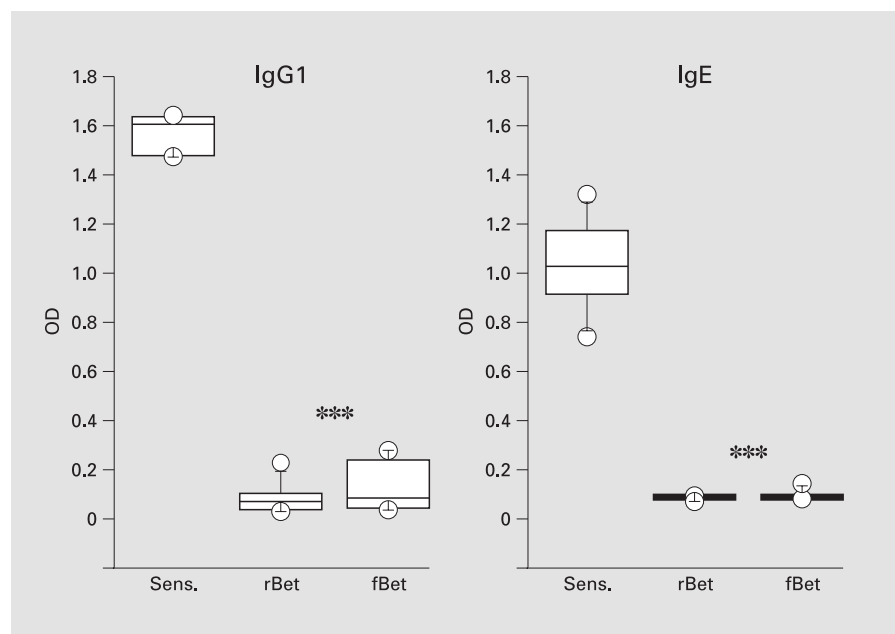
Using a murine model of birch pollen allergy we recently demonstrated that mucosal application of rBet v 1 prior to sensitization led to the suppression of allergic immune responses in vivo and in vitro [8]. In the present study we studied the effects of intranasal pretreatment with hypoallergenic Bet v 1 fragments, in comparison to complete rBet v 1, on the immune responses and airway inflammation/reactivity.

Mice intranasally pretreated with complete rBet v 1 or with Bet v 1 fragments displayed significantly lower allergen-specific IgG1 and IgE antibodies than the sensitized control mice (fig. 1). In accordance with the reduced antibody levels, intranasal pretreatment with complete rBet v 1 and Bet v 1 fragments led to inhibition of cutaneous hypersensitivity reactions (data not shown). In order to investigate whether airway inflammation could be influenced by the mucosal pretreatment with the complete molecule and the fragments, IL-5 production was measured in lung cell cultures and eosinophils determined in bronchoalveolar lavage (BAL) as previously described [10].

IL-5 levels were more than 16 times lower in lung cell cultures of Bet v 1 and Bet v 1 fragment-pretreated mice compared to the sensitized controls. The IL-5 suppression was associated with a reduced eosinophilic infiltration in the BAL of both rBet v 1 and Bet v 1 fragment-pretreated mice. In a subsequent study, using the single Bet v 1 fragments (aa 1–74 or aa 76–160) for mucosal tolerance induction, we noted that the presence of the immunodominant T cell epitope, located in the second fragment (aa 76–160), is crucial for successful tolerance induction, since only the pretreatment with the second fragment but not the first fragment led to inhibitory effects as described.

Airway hyperresponsiveness, a hallmark of allergic asthma, is associated with increased levels of IgE, IL-5

Fig. 1. Allergen/specific serum IgG1 and IgE antibody levels. Bet v 1-specific IgG1 and IgE antibody levels were measured by ELISA. Results are shown for sensitized mice (Sens.), mice intranasally treated with rBet v 1 prior to sensitization (rBet) and mice pretreated with Bet v 1 fragments (fBet). Box plots display optical density (OD) values: each box represents the interquartile range containing 50% of values. The line across the box indicates the median. The whiskers extend from the box to the highest and lowest values, the circles indicate outliers. Mann-Whitney U test, *** $p < 0.001$.



and eosinophilia [11]. Accordingly, sensitized mice displayed airway hyperresponsiveness (AHR) upon methacholine challenge (fig. 2). Intranasal pretreatment with the complete Bet v 1 inhibited the development of AHR, which correlated well with the suppressed IgE, IL-5 and reduced eosinophils in these animals (fig. 2). To our knowledge this is the first study showing that AHR can be inhibited by mucosal tolerance induction. Whether mucosal pretreatment with the Bet v 1 fragments also leads to prevention of AHR is currently under investigation.

In conclusion, our results indicate that mucosal tolerance induction with recombinant allergens and genetically produced hypoallergenic derivatives could be a convenient and safe treatment strategy against allergic diseases.

Acknowledgments

This study was supported by grants from the 'Jubiläumsfonds der österreichischen Nationalbank' and the European Science Foundation.

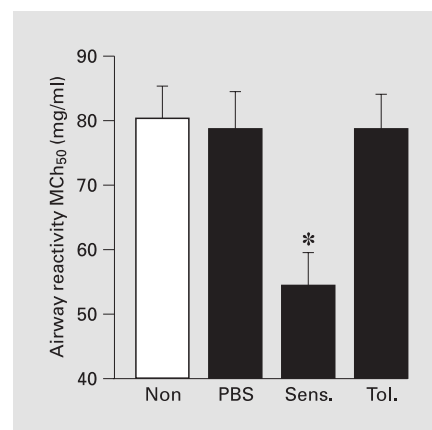


Fig. 2. Airway responsiveness. Measurements of airway responsiveness as assessed by head-out body plethysmography. The concentration of methacholine that caused a 50% reduction in expiratory airflow (MCh_{50} , mg/ml) was determined for untreated mice (Non), sham-treated mice (PBS), sensitized mice (Sens.) and mice intranasally pretreated with rBet v 1 prior to sensitization (Tol). Means of 12 individual measurements are displayed. Error bars indicate SEM. * $p < 0.05$ as determined by Mann-Whitney U test.

References

- 1 Ebner C, Siemann U, Bohle B, Willheim M, Wiedermann U, Schenk S, Klotz F, Ebner H, Kraft D, Scheiner O: Immunological changes during specific immunotherapy of grass pollen allergy: Reduced lymphoproliferative responses to allergen and shift from Th2 to Th1 in T cell clones specific for Phl p1, a major grass pollen allergen. *Clin Exp Allergy* 1997;27:1007–1015.
- 2 Ferreira F, Hirtenlehner K, Jilek A, Godnick-Cvar J, Breiteneder H, Grimm R, Hoffmann-Sommergruber K, Scheiner O, Kraft D, Ebner C: Dissection of immunoglobulin E and T cell reactivity of isoforms of the major birch pollen allergen Bet v 1: Potential use of hypoallergenic isoforms for immunotherapy. *J Exp Med* 1996;183:599–609.
- 3 Vrtala S, Hirtenlehner K, Vangelista L, Pastore A, Eichler H, Sperr R, Valent P, Ebner C, Kraft D, Valenta R: Conversion of the major birch pollen allergen, Bet v 1, into two nonanaphylactic T cell epitope-containing fragments; candidates for a novel form of specific immunotherapy. *J Clin Invest* 1997;99:1673–1681.
- 4 van Hage-Hamsten M, Kronquist M, Zetterström O, Johansson E, Niederberger V, Vrtala S, Grönlung H, Valenta R: Skin test evaluation of genetically engineered hypoallergenic derivatives of the major birch pollen allergen, Bet v 1: Results obtained with a mix of two recombinant Bet v 1 fragments and the rBet v 1 trimer in a Swedish population before the birch pollen season. *J Allergy Clin Immunol* 1999;104:969–977.
- 5 Fanta C, Bohle B, Hirt W, Siemann U, Horak F, Kraft D, Ebner H, Ebner C: Systemic immunological changes induced by administration of grass pollen allergens via the oral mucosa during sublingual immunotherapy. *Int Arch Allergy Immunol* 1999;120:218–224.
- 6 Weiner HL: Oral tolerance for the treatment of autoimmune diseases. *Annu Rev Med* 1997;48:341–351.
- 7 Lowrey J, Savage J, Palliser D, Corsin-Jimenez M, Forsyth L, Hall G, Lindey S, Stewart G, Tan K, Hoyne G, Lamb J: Induction of tolerance via the respiratory mucosa. *Int Arch Allergy Immunol* 1998;116:93–102.
- 8 Wiedermann U, Jahn-Schmid B, Bohle B, Repa A, Renz H, Kraft D, Ebner C: Suppression of antigen-specific T and B cell responses by intranasal or oral administration of recombinant Bet v 1, the major birch pollen allergen, in a murine model of type I allergy. *J Allergy Clin Immunol* 1999;103:1202–1210.
- 9 Neuhaus-Steinmetz U, Glaab T, Daser A, Braun A, Lommatzsch M, Herz U, Kips J, Alarie Y, Renz H: Sequential development of airway hyperresponsiveness and acute airway obstruction in a mouse model of allergic inflammation. *Int Arch Allergy Immunol* 2000;121:57–67.
- 10 Jahn-Schmid B, Wiedermann U, Bohle B, Repa A, Kraft D, Ebner C: Oligodeoxynucleotides containing CpG motifs modulate the allergic immune response of BALB/c mice to Bet v 1, the major birch pollen allergen. *J Allergy Clin Immunol* 1999;104:1015–1023.
- 11 Eum SY, Haile S, Lefort J, Huerre M, Vargaftig BB: Eosinophil recruitment into the respiratory epithelium following antigenic challenge in hyper-IgE mice is accompanied by interleukin 5-dependent bronchial hyperresponsiveness. *Proc Natl Acad Sci USA* 1995;92:12290–12294.

Copyright: S. Karger AG, Basel 2001. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.